

Report No:11539/15/2573

TEST NUMBER ISSUED BY EUROFINS PRODUCT TESTING SPAIN SL

CUSTOMER IDENTIFICATION

NAME: RIO SAGRADO SL

ATT: EDUARDO RODRIGUES do CARMO

ADDRESS: PI As Gandaras Plot 204

LOCATION: 36400 Porrino (Pontevedra)

PRODUCT IDENTIFICATION DATA

Determination of Biodegradability according to OECD 302B

Reference: 14006995

Remarks:

F. Reception: 02/06/15

LAB PHYSICAL AND CHEMICAL TESTS : Start: 20/03/15. Finish:21/04/15

REQUESTED MATTER:

Determination of the Biodegradability according to OECD Standard 302B of a liquid sample sent by the petitioner.

The sample is referenced as SAMPLE FL-02

The Eurofins company has referenced this sample as 14006995.

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The results obtained only attest to the annotated sample

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SUMMARY:

The Biodegradability Determination has been made after 28 days, of the sample referenced 14006995. For the Determination of Biodegradability, the MO 18 operating method has been followed based on the OECD method 302B (1992).

Biodegradation is defined as the decomposition of an organic compound by the biological action of microorganisms, which result in the disappearance of the original molecular structure and the formation of smaller organic molecules. The results are expressed as a percentage of biodegradation at 28 days.

Biodegradability has been determined at two COT concentrations of the present sample: 50 and 150 mg COT/L.

The result obtained in this test indicates that sample 1500980 shows a biodegradation of 93% at the initial concentration of 50 mg COT/L, and a biodegradation of 87% at the concentration of 150 mg COT/L, at 28 days testing.

OBJECTIVE OF THE STUDY

The objective of the study is to determine the biodegradability of the test substance or product at 28 days.

It is based on a known concentration of the test substance (50-400mg COT/L) and the monitoring is carried out by means of the analysis of Total Dissolved Organic Carbon (COT) at intervals over a 28 day period.

TEST CONDITIONS

- Test duration 29 days
- Temperature 22±2° C
- Photoperiod: Diffused light
- Description of the test design:
 - Test containers: 5lt conical flasks
 - Test volume used: 2 litres
- No of test vessels per concentration: 1
- Initial test concentrations: 50mg/l of COT, 150mg/l of COT

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- Reference substance: Diethylene glycol
- Inoculum: 1g activated sludge dry matter Urban scrubber/L
- Composition of Culture Medium:

Solution 1

KH_2PO_4	8,5 g/L H_2O
K_2HPO_4	21,75 g/L H_2O
$\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$	33,4 g/L H_2O
NH_4Cl	0,5 g/L H_2O

Solution2

$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	36,4 g/L H_2O
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Solution3

$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	22,5 g/L H_2O
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Solution4

$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	0,25 g/L H_2O
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Add to 1000ml deionized water:

Solution 1	10ml
Solution 2	1ml
Solution 3	1ml
Solution 4	1ml

Test Development

800ml of mineral medium are introduced into 5lt flasks and sufficient volumes of the stock solutions of the substance (problem and reference) are added to obtain a substance concentration equivalent to the desired mg/COT/L

Taking into account 10.3572g/l of product has a COT value of 92.16mg/L

- A solution of the product of 11.2g/l is made to obtain a COT value of 50mg/L
- A solution of the product of 33.7g/l is made to obtain a COT value of 150mg/L

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The pH is adjusted to 6.5-8.0

- Flasks are inoculated with activated sludges so that a concentration of 1g dry matter/L is obtained
- A control of the inoculum is prepared with the mineral medium but without a test or reference substance
- All flasks are flushed at 2lt with mineral medium and after shaking a sample of each flask is taken to determine the initial concentration of total dissolved organic carbon (TOC)
- Then the vessels are placed in continuous aeration
- Throughout the test the COT concentrations in each flask are determined by time intervals of 2-3 days. For each determination only the minimum necessary volume of problem suspension is taken
- Before taking the samples the losses that have occurred through evaporation of the test medium must be compensated by adding the amount of dilution water
- Before taking the samples the culture medium must be well shaken to make sure the material that has adhered to the walls is dissolved or re-suspended
- Filter by membrane immediately after taking the sample
- The filtered samples are analysed by a COT analyser

RESULTS

The test is carried out on February 16 and April 17 2015. The tests attached in this report have been carried out by our collaborating laboratory "Center de Recerca I Innovation in Toxicology of the UPC" with report number 7527/201.15b laboratory accredited by the CIDEM of the Generalitat de Catalunya. Registered in the Register of Agrifood laboratories and recognised by the Department of Agriculture Livestock and Fisheries, and registered in the registry of laboratories of Environmental and Food Public Health of the Generalitat of Catalunya and certified according to UNE-EN-ISO 9001:2001

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The biodegradation results (28 days) obtained are represented in Table 1 and in the following graphs

%Biodegradation

TIME	Dietilenglicol	50mg/L	150mg/L
0hr	0	0	0
3hr	0,7	6,0	0,6
Day 3	3,8	70,6	62,5
Day 5	27,1	76,5	64,7
Day 7	54,6	79,1	66,9
Day 10	79,2	82,3	72,9
Day12	84,1	86,8	76,5
Day 18	97,0	91,7	84,7
Day 21	100	87,1	84,9
Day 24	100	88,3	85,3
Day 26	100	90,9	86,2
Day 28	100	93,0	87,0

Table 1 Percentage of Biodegradation of the sample over time

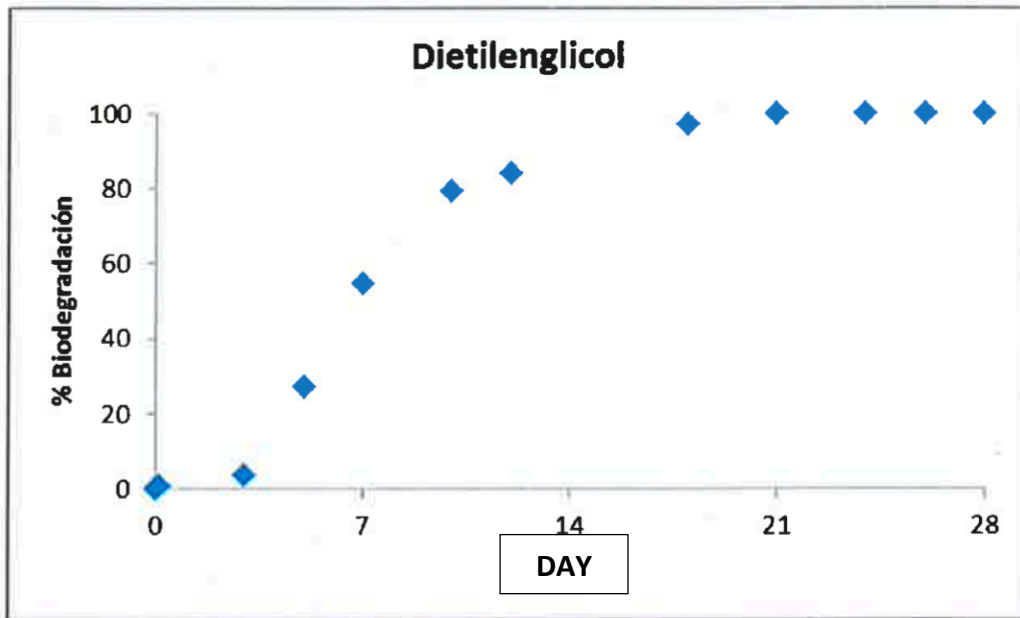
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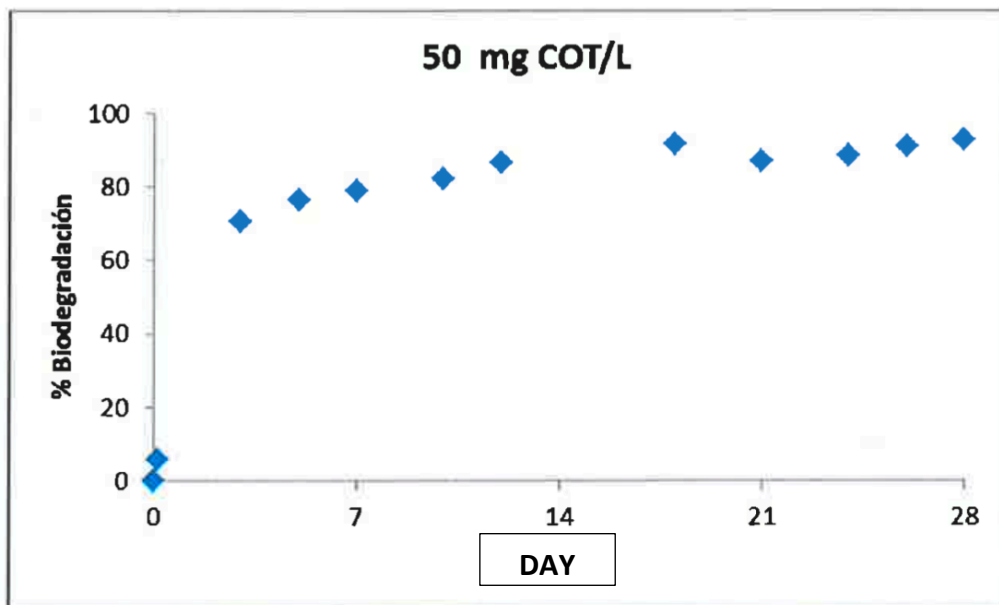
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Graphics:

a) Biodegradation of the reference substance (Dietilenglicol)



b) Biodegradation of the test sample b.1
Initial concentration TOC: 50mg/L

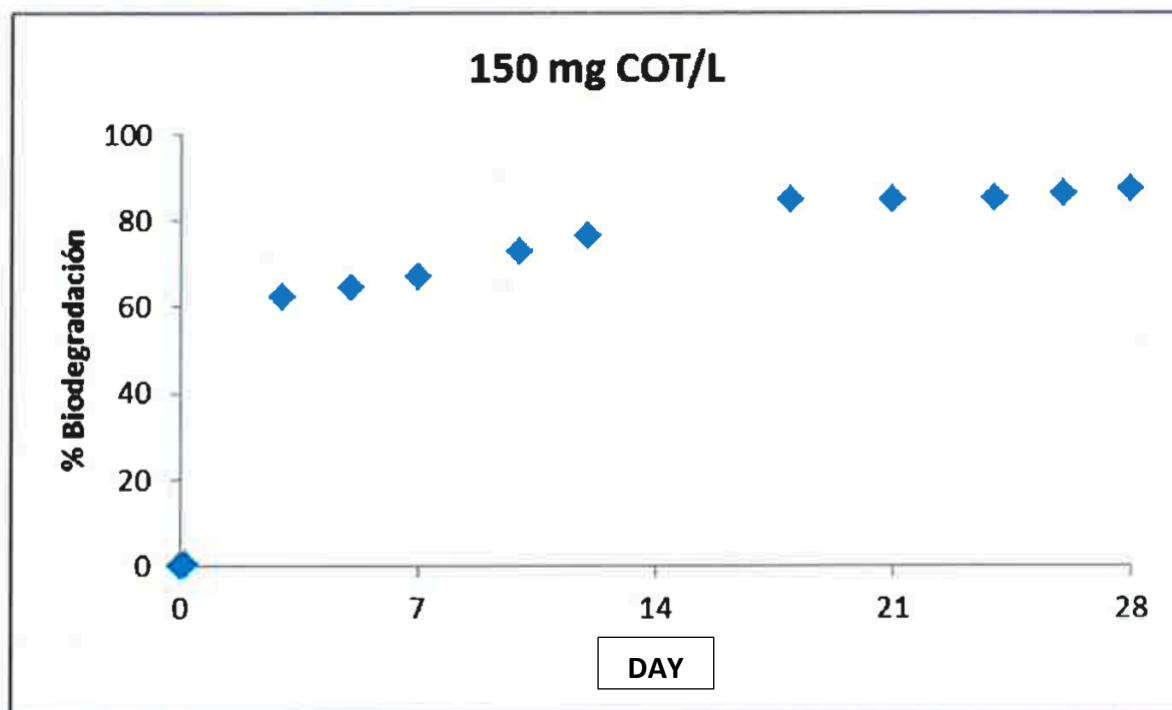


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b2) Biodegradation of the test sample b.2
Initial concentration TOC: 150mg/L



RESULT

The percentage of biodegradation at 28 days of the material presented with the reference FL-02 is:

Initial concentration 50mg COT/L: Bio_{28 days} (%) = 93%

Initial concentration 150mg COT/L Bio_{28 days} (%) = 87%

Validation

The validation criteria for this test is:

The percentage degradation of the product reaches 70% before 14 days

References:

1. OECD Guideline for Testing of Chemicals 302B (1992): "Inherent Biodegradability: Zahn-Wellens?EMPA Test
2. MO 18 v.01 "Biodegradabilidad inherente. Test Zahn-Wellens(EMPA)

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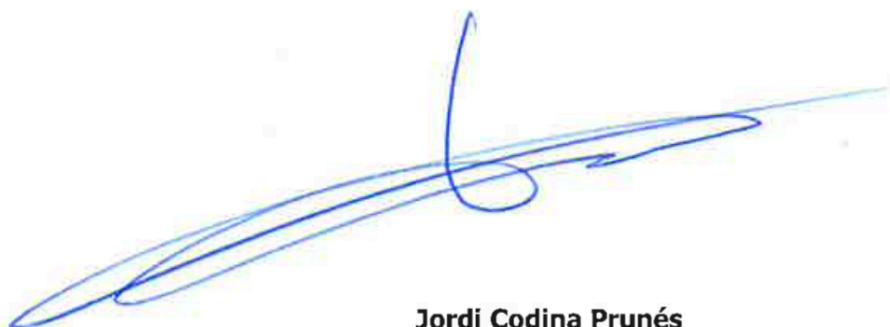
Conclusions:

The study consists of the determination of Biodegradability, which has been carried out according to the MO 18 operating method based on the OECD 302B method, Zahn-Wellings Test.

The FL-02 test sample has a biodegradability of 93% and 87% at the initial two concentrations of COT tested (50 and 150mg/L respectively at 28 days of testing).

The following table summarises the conclusions.

REFERENCE		Biodegradation 28 DAYS
FL-02	50 mg COT/L	93
	150 mg COT/L	87



Jordi Codina Prunés

**Responsable Unidad Confort Ambiental
Bellaterra, 21 de abril de 2015**

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